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SYSTEMATICS OF <u>AEDES</u> MOSQUITO PROJECT FINAL REPORT

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Wayne N. Mathis

January 1985

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND Fort Detrick, Frederick MD 21701-5012

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Research continues on the arbovirus vector groups of the subgenus <u>Stegomyia</u>, genus <u>Aedes</u>, of the Afrotropical Region.

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SUMMARY

The Systematics of Aedes Mosquito Project (SAMP), a cooperative venture between the Smithsonian Institution and the U.S. Army Medical Research and Development Command, conducts biosystematic research on mosquitoes of medical importance to the Army. SAMP fulfills these objectives by performing biosystematic studies on important groups of aedine vectors of arboviruses. SAMP provides information on potential vectors for the guidance of military field research teams and other governmental agencies and prepares monographs and technical papers, which summarize data on the ecology, taxonomy and medical importance of these vectors in Africa. In addition, SAMP performs curation and research on the national collection of mosquitoes at the National Museum of Natural History (NMNH), Smithsonian Institution.

Research continues on the arbovirus vector groups of the subgenus <u>Stegomyia</u>, genus <u>Aedes</u>, of the Afrotropical Region under a separate grant.

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INTRODUCTION

Biosystematic studies, which lead to the precise identification of vectors, are fundamental to any investigation of epidemiology and to the planning of control or eradication. These studies enable recognition of the vector(s); further study of the ecology and habits of the vectors; and effective diffusion of information about vectorial capacity, resistance to insecticides, geographic distribution, etc. Many instances of failure to control diseases resulting from vector-borne pathogens can be traced to neglect of this aspect of research in entomology.

The Systematics of Aedes Mosquito Project (SAMP) was developed to perform biosystematic research on medically important mosquitoes to meet the U.S. Army Medical Research and Development Command's requirements for accurate identification of actual or potential mosquito vectors of pathogens of man in Thus, SAMP is able to respond to these needs and the resources of the project are used to accomplish these requirements. The research was accomplished by 1 professional entomologist plus the principal investigator. The work was supplemented by 4 professional entomologists from the Walter Reed Biosystematics Unit (WRBU) on assignment to the Smithsonian. addition, upon request, SAMP assists various military and other medical entomologists in biosystematic studies of medically important mosquitoes. This level of support may range from furnishing entomologists with keys, necessary literature, and other identification guides, to the loan of specialized collecting and rearing equipment, which cannot be obtained from other sources. Such support has proven invaluable to all concerned, as the Smithsonian Institution has received extremely worthwhile material from these entomologists.

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REVIEW OF PROGRESS FOR THE PERIOD August 1, 1983 - July 31, 1984 (Dr. Yiau-Min Huang)

1. Biosystematic studies on Aedes

Basic to all studies on mosquitoes and to the control of mosquito-borne diseases is the determination of the exact species involved. A detailed knowledge of all life stages and both sexes is usually essential to determine the species. Thus, taxonomic studies should be based on some reared material with their associated larval and pupal skins. This type of material can only be obtained from individual rearings of field-collected larvae or eggs or through rearings of progenies from wild females. There is at present very little of this type of material available for the African fauna. As a result, efforts during the past year was primarily directed toward processing recently acquired specimens, preparing for field work and developing contacts in other countries where the need is greatest for critical material.

During this period the project was begun with work toward a revisionary study of the subgenus <u>Stegomyia</u> (genus <u>Aedes</u>) of the Afrotropical Region as the primary objective. Initial research was focused on studying specimens of the Africanus and Simpsoni species complexes. These complexes contain species that are important vectors of Dengue, Chikungunya, Yellow Fever, Rift Valley Fever and Zika viruses.

During a field trip by the investigator to Cameroon and Kenya in the early part of 1983 numerous specimens, mostly as reared series, were collected. Other specimens were borrowed from the Division of Vector Borne Diseases (DVBD), Ministry of Health, Nairobi, Kenya, during the trip to Cameroon and Kenya. The DVBD houses a major collection of African mosquitoes, mostly from eastern Africa and neighboring territories that is not duplicated elsewhere. There is a large accumulation of material from the last 40 years of European entomological studies in Africa. The collections at the DVBD were carefully studied and identified, and the data from 678 specimens were recorded. A total of 116 of the most important specimens were selected for loan to the Smithsonian for further study.

During the 1940's British researchers in Uganda incriminated Aedes (Stegomyia) simpsoni (Theobald) as one of the primary vectors of Yellow Fever virus in primates, including man, in eastern Africa. Since that time the major medical and public health texts on African arboviral vectors have listed simpsoni as the major endemic vector of primate Yellow Fever virus in Africa. During this same period, however, 2 distinct populations of simpsoni were recognized in Uganda, one man-biting and the other non-man-biting.

Study of "simpsoni" specimens in collections of the DVBD, which were made primarily in Uganda, Kenya, Tanzania and Malawi, has revealed that the species from which Mahaffy et al. (1942) isolated Yellow Fever virus is in fact Aedes (Stegomyia) bromeliae (Theobald) and is the common man-biting member of the complex in eastern Africa. The species previously called simpsoni in Kenya, Tanzania, and Malawi is also bromeliae. In Uganda, specimens from Bwamba and Kampala counties, which were identified as simpsoni, are bromeliae, and the specimens from Karamoja Kaabong, identified as simpsoni, are bromeliae and lilii (Theobald). However, 91% of all specimens of the complex examined were bromeliae and only 9% of the specimens were lilii, which may be the non-man-biting member of the complex.

Examination of all available specimens at SAMP, including those on loan from many museums in Europe and Africa, has revealed that "simpsoni" is actually a complex of at least 3 species. Aedes simpsoni is found to be restricted to South Africa, and is not known to be involved in the transmission of arboviruses. Aedes bromeliae is a common species that is widespread throughout the Afrotropical Region, while Aedes lilii is also widely distributed across Africa, but does not appear to be as common.

As a result of these studies, 2 manuscripts have been written. The first paper concerns the description, including illustrations, of a new species of African Stegomyia that is closely related to the Simpsoni complex. This paper is based on a single female that was collected while it fed on a man in Kenya. Because only one specimen is available, this paper is being held, pending collection of more material. The second paper, entitled "Aedes (Stegomyia) bromeliae (Theobald), the Correct Identity of the Sylvan Yellow Fever Vector in East Africa," is now being reviewed. The purpose of this paper is to clarify the identity of the common Yellow Fever vector of eastern Africa, Aedes (Stegomyia) bromeliae Theobald, and to confirm an earlier report (Huang 1979) that what was previously called Aedes (Stegomyia) simpsoni for the Afrotropical Region is a complex of at least 3 species, viz. simpsoni (Theobald), lilii (Theobald), and bromeliae. This paper will be submitted for publication in the Journal of Medical Entomology in early 1985. During the course of this work, more than 1261 specimens (including 257 male and female terminalia) were examined and over 50 important references on simpsoni were reviewed.

Examination of the "africanus" specimens, which were recently brought back on loan from the DVBD, indicates that the species previously called "africanus" in eastern Africa--Uganda (Entebbe), Ethiopia (Kaffa Prov.), and Kenya--is a different species. Aedes africanus was incriminated as a vector of Yellow Fever in the forest cycle in Uganda (Haddow et al., 1948) and during a major epidemic in Ethiopia in the 1950's and 1960's. This species was considered the primary link between the sylvan and human phases of the Yellow Fever cycle in Ethiopia (Neri et

al., 1968). The probability that this is a new species, not A. africanus, will cause a major change in vector concepts regarding the sylvan Yellow Fever cycle in Africa. A detailed study of the Africanus subgroup is under way. However, much more material from both eastern and western Africa will be needed before final decisions and a revision of the subgroup can be made. A manuscript describing a new species of African Stegomyia in the Africanus complex will be submitted for publication in Mosquito Systematics during 1985.

In addition to the above, a study was also initiated on Aedes (Neomelaniconion) lineatopennis (Ludlow). This species has been incriminated as a vector of Rift Valley Fever by McIntosh (1972) in Zimbabwe, by Davies and Highton (1980) in Kenya, and by McIntosh et al. (1980) in South Africa. It is not certain. however, if the so-called "lineatopennis" from Africa is conspecific with the type from the Philippines. To clarify the status of African "lineatopennis," especially in view of current research on this species as a vector of Rift Valley Fever, study will be focused on reassessment of the conspecificity of the African populations with those in the Philippines. This study is in response to a request from LTC Charles L. Bailey, U.S.Army Medical Research Institute for Infectious Disease for assistance, and in support of ongoing U.S.Army Medical Research and Development Command research efforts in Africa. A preliminary examination of all available specimens of A. lineatopennis is underway and will continue under a new grant.

Considerable time was devoted to preparation for and conducting field work in western Africa, and a very successful field trip was made to Senegal and Sierra Leone, from April 16 to June 27, 1984. The objectives of this trip were: (1) to study the Africanus subgroup and other African Stegomyia specimens at the Institut Pasteur, Dakar, Senegal; (2) to discuss with Dr. M. Cornet, Office de la Recherche Scientifique et Technique Outre-Mer, in Dakar, future collaborative arrangements for collecting specimens in western Africa; (3) to undertake field studies in Sierra Leone, collecting and individually rearing topotypic and other critical material, obtaining biological and ecological information, with primary emphasis on potential aedine arboviral vectors, with secondary emphasis on species of Anopheles and Culex.

The collections at the Institut Pasteur de Dakar, Senegal, were carefully studied, identified, and the data recorded. A total of 386 adults, 86 slides of larval skins, and 19 vials of larvae. larval and pupal skins, of the most important Aedes (Stegomyia) species were selected for loan to the Smithsonian for further study.

In Sierra Leone, 4 localities in the western area and southern province were visited for field collections. A total of 208 collections were made: 92 collections from Tiwai Island, 56 collections from Fourah Bay College area, 48 collections from

Njala University College area and 12 collections from Kasewe Forest Reserve. Special effort was made to collect immatures. particularly the larval stages in the natural habitat and to carry out individual rearings to obtain adults with associated larval and pupal skins. The immature stages are essential for clarifying the identity of a species and resolving species complexes. A total of 114 immature collections were made: collections from Tiwai Island (tree hole, stump hole, cut bamboo, bamboo stump, rock pool, ground pool), 10 collections from Fourah Bay College (tree hole, bamboo stump), 41 collections from Njala University College (tree hole, leaf axil) and 12 collections from Kasewe Forest Reserve (tree hole, stump hole, ground pool, fallen leaf). A total of 76 oviposition traps (plastic bottle, beer can) were placed in the field for obtaining eggs. Eggs collected by this method have been hatching at SAMP, Smithsonian, since July 1984. In addition, a total of 18 adult collections (biting. resting) were made from Tiwai Island, Fourah Bay College and Njala University College. Locality and ecological data were recorded on standard collection forms and are on file with the Smithsonian mosquito collection.

A total of 1776 adults with associated larval and/or pupal skins, belonging to 7 genera (Aedes, Anopheles, Culex, Eretmapodites, Malaya, Toxorhynchites and Uranotaenia) were obtained from Sierra Leone. All of the adults have been identified and sorted to genus, subgenus, species complex or species. At least 85% of the total are Aedes and Culex, and a good series of all stages (male, female, larva and pupa) were obtained for each species of Aedes and Culex collected. This material will be extremely valuable for current studies at SAMP and Walter Reed Biosystematics Unit (WRBU).

Work on the Sierra Leone Stegomyia specimens, which were collected during the recent field trip there, is under way. It is hoped that study of this material will resolve some of the taxonomic problems related to the Africanus, Luteocephalus, Simpsoni and Dendrophilus species complexes, as well as problems associated with species such as <u>fraseri</u> (Edwards)/<u>blacklocki</u> Evans, <u>apicoargenteus</u> ssp. <u>denderensis</u> Wolfs and <u>aegypti</u> ssp. formosus (Walker).

2. Curatorial Activities

During this period new material consisting of 864 adults (including 564 individual rearings with associated larval and/or pupal skins) of African Stegomyia were acquired from the following sources: (1) 34 adults, 31 larval skins, 34 pupal skins from Mrs. M. Coetzee, Tzaneen, South Africa; (2) 239 adults with larval and pupal skins from Mr. D.L. Theron, National Institute for Tropical Diseases (NITD), Tzaneen, South Africa; (3) 84 adults, 47 larval skins, 69 pupal skins (Senegal), from CPT M.E. Faran et al., USAMRIID; (4) 136 adults with larval and pupal skins (Somalia) and 31 adults with larval and pupal skins (Sudan), from LCDR J.H. Zimmerman, USN, Medical Zoology Department, NAMRU-3, FPO New York 09527; (5) 338 adults, 86 slides of larval skins and 19 vials of larvae, larval and pupal skins (Senegal), from Dr. M. Cornet, Institut Pasteur de Dakar, Senegal. In addition, 294 adults, 144 larval and/or pupal skins, and 6 lots of eggs of other subgenera of Aedes (Aedimorphus, Diceromyia, Finlaya, Mucidus, Neomelaniconion) from Senegal were received from CPT M.E. Faran et al., USAMRIID and 48 adults and 2 lots of eggs of Aedes (Diceromyia) from Senegal were received from Dr. M. Cornet, Institut Pasteur de Dakar, Senegal.

3. Other Activities

(1) Identified 371 Aedes (Aedimorphus, Diceromyia, Finlaya and Stegomyia) mosquitoes from Senegal, Kenya, Somalia, Sudan and Trinidad, W.I. for CPT M.E. Faran, USAMRIID; LCDR J.H. Zimmerman, USN, Medical Zoology Department, NAMRU-3; and Dr. A.B. Knucsen, Caribbean Epidemiology Centre (PHO/WHO), Port-of-Spain, Trinidad, W.I.; (2) Translated 4 Chinese papers regarding Anopheles, Aedes, Uranotaenia and fossil mosquitoes (Asioculicus Hon, n. gen. and 2 new species) for Dr. R.A. Ward, WRAIR; (3) Reviewed 2 manuscripts written by others for publication.

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- Appendix 1: LIST OF SAMP CONSULTANTS
- Dr. Michel Cornet, Institut Pasteur de Dakar, B.P. 220, Dakar, Republique du Senegal. African <u>Aedes</u>

- Or. George B. Craig, Jr., Department of Biology, University of Notre Dame, Note Dame, Indiana 46556. Aedes genetics
- Dr. Max Germain, ORSTOM, 70-74 route d'Aulnay, 93140 Bondy, France. African Aedes
- CPT Ralph E. Harbach, Ph.D., Walter Reed Biosystematics Unit, NHB Stop 165, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560. Culex
- LTC Bruce A. Harrison, Ph.D., Manager, Walter Reed Biosystematics Unit, NHB Stop 165, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560. Old World Anopheles and Southeast Asian mosquitoes
- Professor Kenneth L. Knight, North Carolina State University, Raleigh, North Carolina 27650. Aedes (Finlaya)
- Dr. Kenneth J. Linthicum, U.S Army Medical Research Unit-Kenya, Box 401, APO New York 09675. African Aedes, Neotropical Anopheles
- Dr. Botha de Meillon, Philadelphia, Pennsylvania. African Culicidae and Ceratopogonidae
- Dr. J. Mouchet, ORSTOM, Head, Department of Entomology, Bondy, France. Culicidae.
- Dr. Lewis T. Nielsen, Biology Department, University of Utah, Salt Lake City, UT 84112. North American Aedes (Ochlerotatus)
- Mr. E L. Peyton, Walter Reed Biosystematics Unit, NHB Stop 165, National Museum of Natural History, Smithsonian Institution, Washington, D.C. 20560
- Dr. Shivaji Ramalingam, University of Malaya, Kuala Lumpur, Malaysia.

 <u>Topomyia, Malaya, Armigeres</u> and Malaysian Culicidae
- Mrs. Rampa Rattanarithikul, Department of Medical Entomology, U. S. Army Component, Armed Forces Research Institute of Medical Sciences, APO San Francisco 96346. <u>Aedes (Finlaya)</u> and Thailand Culicidae
- LTC John F. Reinert, Ph.D., Headquarters USAMRDC, SGRD-DPM, Ft. Detrick, MD 21701. Aedes subgenera
- Dr. Ronald A. Ward, Department of Entomology, Walter Reed Institute of Research, Washington, DC 20012. New World mosquitoes

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Appendix 2: Mosquitoes Collected in Sierra Leone, 1984 (SAMP Acc. 1093)

At least 40 species belonging to 7 genera (Aedes, Anopheles, Culex, Eretmapodites, Malaya, Toxorhynchites and Uranotaenia) were collected from Sierra Leone. These included 2 new species (1 in Aedes and 1 in Culex), topotypic specimens for 5 species Aedes apicoannulatus (Edwards), Aedes aegypti ssp. formosus (Walker), Aedes africanus (Theobald), Aedes blacklocki Evans, and Uranotaenia fusca Theobald, 6 new species for Sierra Leone Aedes flavicollis Edwards, Aedes denderensis Wolfs, Culex garioui Bailly-Choumara and Rickenbach, Malaya taeniarostris (Theobald), Toxorhynchites erythrurus (Edwards) and Uranotaenia nigromaculata Edwards, 2 subspecies Aedes apicoargenteus ssp. denderensis Wolfs, and Toxorhynchites brevipalpis ssp. conradti Grunberg, that should be elevated to full species status, and 1 current synonym (Aedes blacklocki Evans) that should be recognized as a distinct species.

```
Aedes (Aedimorphus) apicoannulatus (Edwards) -- (topotypic specimens)
         minutus (Theobald)
         simulans (Newstead & Carter)
         tarsalis (Newstead)
         vittatus (Bigot)
     (Diceromyia) flavicollis Edwards -- (new record for Sierra Leone)
     (Finlaya) longipalpis (Grunberg)
     (Pseudarmigeres) argenteoventralis (Theobald)
     (Stegomyia) aegypti (Linnaeus)
              ssp. <u>formosus</u> (Walker) -- (topotypic specimens) <u>africanus</u> complex -- (topotypic specimens)
                   apicoargenteus (Theobald)
              ssp. denderensis Wolfs -- (new record for Sierra Leone, should be
                                           elevated to specific status)
Aedes (Stegomyia) bromeliae (Theobald)
                   dendrophilus complex
                   fraseri (Edwards)
                   blacklocki Evans -- (topotypic specimens, currently a
                                          synonym, but should be elevated to
                                          species status).
                   lilii (Theobald)
                   luteocephalus complex
Anopheles (Cellia) gambiae complex - 1 sp.
Culex (Culex) decens Theobald
          invidiosus Theobald
          nr. schwetzi Edwards - 1 n.sp.
          thalassius Theobald
       (Culiciomyia) macfiei Edwards
                     nebulosus Theobald
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- (Eumelanomyia) albiventris Edwards

 garioui Bailly-Choumara and Rickenbach (new record for Sierra Leone)
- (Lutzia) tigripes De Grandpre and De Charmoy

Eretmapodites chrysogaster Graham oedipodius Graham

semisimplicipes Edwards

Malaya taeniarostris (Theobald) (new record for Sierra Leone)

Toxorhynchites (Toxorhynchites) brevipalpis

ssp. <u>conradti</u> Grunberg
" <u>erythrurus</u> (Edwards) (new record for Sierra Leone)

phytophagus (Theobald)

<u>Uranotaenia</u> (<u>Pseudoficalbia</u>) <u>fusca</u> Theobald (topotypic specimens) <u>nigromaculata</u> Edwards (new record for Sierra Leone)

ornata Theobald

The <u>Culex</u> were identified by CPT (Dr.) R.E. Harbach, Walter Reed Biosystematics Unit.

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